

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Yuuki Tsutsui, *et al.*

Application No.: 10/674,581

Filed: September 29, 2003

For: IMMUNE RESPONSE
INDUCTION METHOD

Customer No.: 20350

Confirmation No. 5398

Examiner: Bruce D. Hissong

Technology Center/Art Unit: 1646

DECLARATION

I, Yuuki Tsutsui, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. I am a citizen of Japan, and I have received the degree of master in pharmacology from Nagoya City University. My *Curriculum Vitae* is attached as Exhibit 1.
2. I have been employed by Astellas Pharma Inc. (formally Yamanouchi Pharmaceutical Co., Ltd., the assignee of the present invention) since 1995.
3. I have been engaged in research and development regarding pharmaceutical preparations since joining Astellas.
4. I am a co-inventor of the inventions described in the specification of the above-identified application (hereinafter, referred to as the "present application").
5. As described herein, a study was undertaken to examine the efficacy of interferon α ("IFN α ") as a mucosal adjuvant, which is a technological feature of the present

invention, in comparison against interferon β ("IFN β "), as well as cholera toxin B subunit (CTB). IFN β is a cytokine adjuvant and CTB has been reported to be very effective as a mucosal adjuvant. The purpose of the study was to evaluate the characteristics and efficacy of IFN α as a mucosal adjuvant.

6. In comparing IFN α against IFN β , ovalbumin (OVA) was used as a vaccine antigen. The IFN α or IFN β at a dose of 4000U along with the OVA was intra-nasally administered to C57BL mice (8-week old) three times (Day 0, 7, and 14). Blood and nasal mucosa were collected on Day 15, 21 (3w), and 28 (4w) after the first administration Day 0, and the levels of OVA-specific antibodies (IgG and IgA) in each sample were measured by ELISA.

7. In comparing IFN α against CTB, ovalbumin (OVA) was used as a vaccine antigen. IFN α at a dose of 4000U or 1 μ g of CTB along with the OVA was intra-nasally administered to C57BL mice (8-week old) three times (Day 0, 7, and 14). Blood and nasal mucosa were collected on Day 15, 21 (3w), and 28 (4w), and the levels of OVA-specific antibodies (IgG and IgA) in each sample were measured by ELISA.

8. At a dose of 4000U, IFN β and IFN α , both were confirmed to induce comparable levels of circulating antigen-specific IgG (Fig. 1). On the other hand, examination of the antigen-specific IgA excreted from the nasal mucosa confirmed that IFN α surprisingly has a higher antibody-inducing ability compared with IFN β (Fig. 2). These results illustrate that IFN α is a cytokine having an unexpected higher mucosal adjuvant efficacy in comparison with IFN β .

9. In addition, IFN α was also compared against CTB, which is a very effective mucosal adjuvant. The dose of CTB applied was 1 μ g, which has been reported in the literature to exert mucosal adjuvant efficacy. The dose of IFN α applied was 4000U. Both were confirmed to induce comparable levels of circulating antigen-specific IgG (Fig. 3). On the other hand, examination of the antigen-specific IgA excreted from the nasal mucosa confirmed that IFN α has a high antibody-inducing ability. The results illustrate that IFN α has a high mucosal adjuvant efficacy.

10. CTB at high doses is widely known to cause adverse effects such as diarrhea, whereas IFN α is applicable at much higher doses in view of known clinical applications. Thus, in application to humans, it is my scientific opinion that IFN α is expected to be useful as a safer and more practical mucosal adjuvant than CTB.

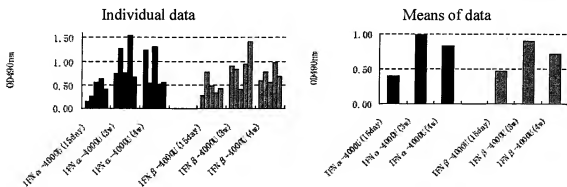


Fig. 1 Results of the comparison between the circulating OVA-specific IgG-inducing abilities of IFN α and IFN β .

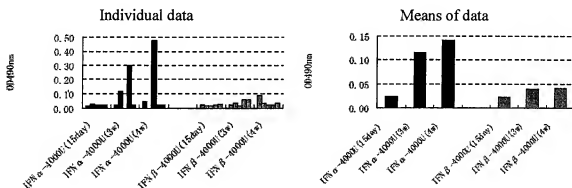


Fig. 2 Results of the comparison between the nasal mucosal OVA-specific IgA-inducing abilities of IFN α and IFN β .

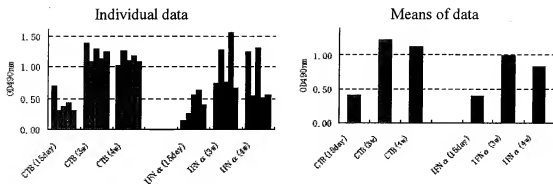


Fig. 3 Results of the comparison between the circulating OVA-specific IgG-inducing abilities of IFN α and CTB.

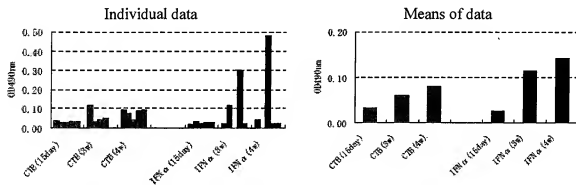


Fig. 4 Results of the comparison between the nasal mucosal OVA-specific IgA-inducing abilities of IFN α and CTB.

The declarant has further nothing to say.

Yuuki Tsutsui

Yuuki Tsutsui

2008. 6. 24

Date

Exhibit 1

Yuuki Tsutsui's *Curriculum Vitae*:

I have received the degree of Faculty of Pharmaceutical Science in March 1993 from Nagoya City University and the degree of Master in Pharmacology in March 1995 from Nagoya City University.